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Viral Infections in Renal Transplant Recipients

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Cytomegalovirus (CMV) infections are common in renal transplant recipients. We studied 23 recipients prospectively to determine whether infections by other herpes-group and non-herpes-group viruses were also present. Sera, obtained at the time of surgery and periodically thereafter, were tested for antibody to CMV, herpes simplex virus (HSV), Epstein-Barr virus (EBV), parainfluenza viruses types 1, 2, and 3, and the viruses of measles and rubella. We found no evidence of an unusual incidence of primary or secondary infection by the non-herpesviruses tested. Rises to CMV, HSV, and EBV antibody titers occurred in 43, 38, and 32% of patients, respectively. All serological rises to herpes-group viruses occurred in patients seropositive at the time of transplantation, with the exception of three patients who experienced primary CMV infections. We conclude that reactivation of all herpes-group viruses tested may occur in transplant recipients. Morbidity was associated only with primary CMV infection.

Primary and reactivation infections with cytomegalovirus (CMV) are common occurrences after transplant surgery and immunosuppression (3, 4). Infection by other herpesviruses such as herpes simplex virus (HSV) and Epstein-Barr virus (EBV) may also be increased (5, 9, 10). This study undertook to compare the rate of infection by the herpesviruses to the rate of infection by some non-herpesviruses. Serum samples obtained before and at intervals after renal transplantation were tested for antibodies to CMV, HSV, EBV, parainfluenza viruses types 1, 2, and 3, and the viruses of measles and rubella. The sera were also examined for the presence of hepatitis B (HbB) antigen. The clinical course of each patient was analyzed in conjunction with the laboratory results and the clinical features pertaining to these viruses. Patients with fever of unknown origin received special attention.

MATERIALS AND METHODS

Study population. The study group (Table 1) consisted of 23 patients who received their renal transplants at the Presbyterian-University Hospital from August 1972 to May 1973. There were 11 males and 12 females. The mean age was 27.3 years, with a range of 8 to 56 years. Preoperative bloods were obtained on all patients and at 2- to 4-week intervals after operation. The range of follow-up was 22 to 282 days. Only one patient was followed for less than 76 days (because of rejection of graft), and the mean follow-up period was 168.5 days.

Patients received 250 mg of methylprednisolone intravenously at the time of operation, 125 mg intravenously daily for 3 days, and 60 mg by mouth daily thereafter. Azathioprine was given by mouth in doses of 2 to 3 mg/kg before the operation and daily thereafter. In three patients, cyclophosphamide was substituted for azathioprine for some time.

Serology. In Pittsburgh, the complement fixation (CF) test for CMV was performed by the microtiter method of Takatsy as modified by Sever (8). We used antigen prepared from CMV strain AD 169 (Microbiological Associates, Inc., Bethesda, Md.). At Yale, the hemagglutination inhibition test was performed for measles, rubella, and parainfluenza viruses, the CF test for HSV and CMV, and the indirect immunofluorescent test for viral capsid antibody to EBV as described previously (1); standard microtiter procedures were used. Automated dispenser and pipetting equipment (Cooke Engineering Co., Alexandria, Va.) were used to improve accuracy. HbB antigen and antibody tests were based on a radioimmunoassay method (Ausria II; Abbott Laboratories, North Chicago, Ill.). A fourfold or greater change in antibody titer was taken as significant.

Clinical analyses. (i) Fever. Fever was defined as 2 or more consecutive days with peak temperatures greater than 37.8°C orally or 38°C rectally. Fever during the first 2 weeks after transplantation was excluded, since it was almost always associated with a mild to moderate degree of rejection. Febrile episodes after this period were categorized as due to (i) rejection, (ii) bacterial infection, (iii) drug reaction, or (iv) unknown origin.

(ii) Rejection. Rejection was defined as an event characterized by a sudden drop in urine output together with rising serum blood urea nitrogen and

TABLE 1. *Infection with CMV*

Patient no.	Age (yr), sex	Follow-up (days)	CMV		CMV antibody		Primary or secondary infection
			Viremia	Viruria	Preop titer	Postop rise	
1	21, M	22	–	–	0	0	
2	8, F	181	–	–	0	0	
3	11, F	267	+	+	+	+	Secondary
4	21, F	250	+	+	0	+	Primary
5	25, F	104	–	–	+	0	
6	25, M	76	–	–	+	0	
7	26, F	154	–	–	0	0	
8	40, M	253	–	–	0	0	
9	13, F	219	+	+	0	+	Primary
10	23, F	83	–	–	+	0	
11	20, M	146	–	+	+	+	Secondary
12	40, F	178	–	–	+	+	Secondary
13	56, M	205	–	+	+	+	Secondary
14	21, M	81	+	+	+	+	Secondary
15	21, F	133	–	–	0	0	
16	28, M	201	–	–	0	0	
17[a]	27, M	282	–	–	+	0	
18	28, M	182	+	+	0	+	Primary
19	43, M	186	–	–	0	0	
20	39, F	187	+	+	+	+	Secondary
21	27, M	144	–	–	+	+	Secondary
22	29, F	160	–	–	0	0	
23	20, F	182	–	–	0	0	
% Positive			26.0	34.7		43.5	
% Positive by one or more tests			43.4				

[a] Second transplant.

creatinine with or without fever and leukocytosis, occurring at an interval after transplantation after there was good urine output.

(iii) Laboratory data. Total and differential leukocytes, total bilirubin, alkaline phosphatase, and lactate dehydrogenase were routinely determined.

RESULTS

Serological. (i) CMV. Significant changes in CF antibody titer to CMV were seen in 11 of the 23 patients studied (47.8%), including one patient in whom a fourfold fall in titer was observed. Three of 12 patients who were initially seronegative showed antibody responses with viremia and viruria (Fig. 1, Table 1) during the study period, and seven of 11 initially seropositive patients showed fourfold or greater rises in titer accompanied by virus isolation in five. Overall this represents a 43% serological response to this antigen (Table 2). Similar results were obtained in duplicate testing at Pittsburgh and at Yale University on these same sera.

(ii) HSV. Of 21 patients tested using HSV CF antigen, 14 were seropositive at the time of transplantation. Of these, eight showed at least fourfold rises in titer (Fig. 2), and HSV was isolated from the urine of one patient (no. 3). One patient had an eightfold fall in titer. None of the seven originally seronegative patients seroconverted. Overall, 38% of patients studied showed antibody rises to HSV.

(iii) EBV. Serological responses to EBV were also seen only in seropositive patients; seven out of 19 (37%) showed fourfold or greater rises in antibody titer to this virus. However, it should be noted that four seropositive patients showed fourfold or greater falls in titer during the study period (Fig. 3). The heterophile antibody titer was measured on 47 sera from 13 patients, including two who showed rises in EBV titer. All sera were negative at 1:10 dilution after guinea pig kidney absorption using horse cells except for one serum at 1:20, a patient with completely negative EBV antibody titers in serial sera; this titer is nondiagnostic.

(iv) Other antigens. Data for rubella, measles, and parainfluenza 1, 2, and 3 are shown on Table 2. Falling titers, seen in 11 cases, were almost as frequent as rising titers against these viral antigens, and the overall frequency of positive serological responses was substantially lower than that seen against the viruses of the herpes group.

(v) Analysis of serological responses. Positive serological responses are summarized in

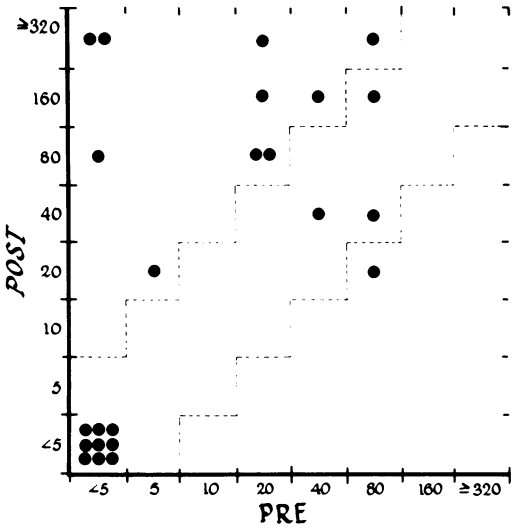

FIG. 1. *Changes in CMV antibody titers. Each point represents a single patient whose titer at the time of transplantation is shown on the abscissa (pre) and whose titer after transplantation is shown on the ordinate (post). If a ≥fourfold increase is followed by a decrease, the peak titer was used. The broken lines are drawn so that changes ≥fourfold lie outside the lines.*

Table 2, in which patients are classified by their initial serological status. Owing to the small number of patients in certain categories, the data were regrouped for statistical analyses by considering the responses to herpesviruses as a group, which were contrasted with responses to the other, non-herpes antigens tested. The regrouped data are shown (all patients, Table 3). Since both numerators and denominators of the fractions shown in Table 2 were summed to obtain the data in Table 3, the numbers in the latter table represent events. Overall, serological rises to herpes-group viruses were seen in 38%, whereas rises in antibody titer to the other viruses tested were seen in only 11%. This difference is highly significant statistically. Further examination of Table 2 leads to the hypothesis that the bulk of the serological responses to the herpes-group viruses except for CMV occurred in seropositive individuals. Therefore, in Table 3 seroconversion in initially seronegative patients is examined separately. For the herpes group, seroconversion occurred on 14% of 22 possible occasions. This represents three apparently primary CMV infections with no evidence of primary infection by EBV or HSV. There were two seroconversions in eight patients initially seronegative for the non-herpes viral antigens tested. The slight difference seen in this table between the herpes group and other viruses is not significant. It is clear that the difference between the herpes group and the other viruses tested lies in the remarkably high overall response (50%) shown by seropositive

TABLE 2. *Serological responses to viral antigens in renal transplant recipients*

Antigen[a]	Initial status[b]	Responses by initial status[c]		All patients	
		Fraction	%	Fraction	%
CMV	Negative	3/12	25	10/23	43
	Positive	7/11	64		
HSV	Negative	0/7	0	8/21	38
	Positive	8/14	57		
EBV	Negative	0/3	0	7/22	32
	Positive	7/19	37		
Rubella	Negative	0/2	0	1/19	5.3
	Positive	1/17	5.9		
Measles	Negative	0/3	0	4/23	17
	Positive	4/20	20		
PI-1	Negative	1/2	50	2/23	8.7
	Positive	1/21	4.8		
PI-2	Negative	1/3	33	2/23	8.7
	Positive	1/20	5		
PI-3	Negative	0/0	-	3/23	13
	Positive	3/23	13		

[a] Abbreviations: PI-1, parainfluenza virus type 1; PI-2, parainfluenza virus type 2; PI-3, parainfluenza virus type 3.

[b] Negative, antibody not detected in serum collected at or about the time of transplant at lowest level tested. Positive, antibody detected in serum collected at or about the time of transplant.

[c] Seroconversion or antibody rise ≥fourfold.

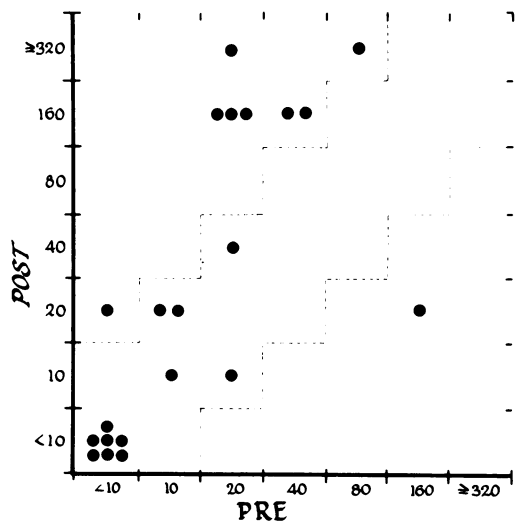

FIG. 2. *Changes in HSV antibody titers. (For details see Fig. 1.) One patient with a pretransplant titer of 1:5 and a post-transplant titer of 1:20 appears in this figure to be a primary case but was not so considered.*

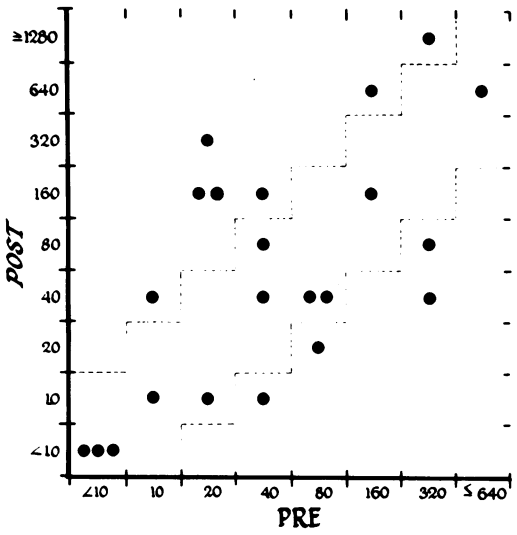

FIG. 3. *Changes in EBV antibody titers. (For details see Fig. 1.)*

patients (Table 3). When this is compared with the combined 9.9% serological response in rubella, measles, and parainfluenza, group viruses, the difference is highly significant.

(vi) Time of serological change in relation in transplantation. Although the serological responses described above occurred after transplantation, it is possible to interpret these results solely in terms of fluctuations in titer unrelated to transplantation and the concurrent immunosuppression. Therefore, the frequency of serological change was examined as a function of time after transplantation (Table 4). It should be noted that the number of responses is not necessarily the same as those shown in Fig. 1 through 3 or Tables 2 and 3. Thus, if a patient showed a significant rise in titer after transplantation and later during the follow-up period showed a significant fall in titers, both events are shown in Table 4. For CMV and HSV, it is reasonably clear that the majority of serological rises tended to fall within a certain period after transplantation. For CMV, the bulk of these rises occurred from 60 to 180 days postoperatively. For HSV there was a sharp peak of serological response from day 30, tapering off to 180 days postoperatively.

With EBV the situation is less clear, since there appears to be an aggregation of both rises and falls in the period from the time of transplantation to 150 days post-transplantation. In this regard, the more subjective aspects of interpreting immunofluorescent antibody titers as compared with CF and hemagglutination inhibition titers should be kept in mind. In Table 4, data from non-herpesvirus antigens are grouped together, since the number of serological changes involved are small. There is some evidence of a period of rising titers after transplantation followed by a period of falling titers from 120 days post-transplantation onward.

(vii) HbB antigen. Sera were also tested for HbB antigen, and 11 of 23 patients (47.8%) showed transient antigenemia, but no consistent changes were observed in any. HbB antibody was detected in only one serum. This serum was collected from patient number 13 at 58 days postoperatively. Six subsequent bleedings from the same patient showed no HbB antibody, nor was antigenemia detected in this patient.

Clinical-serological correlation. (i) Herpetic lesions. There were five patients who developed herpetic-type sores (four oral, one genital). Of these, three were seropositive for antibodies against HSV preoperatively, and all had postoperative rises in titer. Five other patients who had a serological rise did not show any manifestation attributable to HSV. Two patients developed herpetic-like lesions in the absence of serological change: one was seropositive and the other was seronegative for antibody against HSV before the operation. It should be noted that the seronegative patient was only followed 22 days after the operation, at which time the lesion was noted. One patient had herpetic lesions and a rise in titer against EBV, but five had rises and did not have such lesions. Two patients also had herpetic lesions (one genital) accompanied by a rise in titer against CMV, but seven who did not have lesions had rises in titer. The one patient with the genital sores also had a rise in CMV antibody titer.

Thus, there is a suggestive correlation between serological rises against HSV and the development of herpes-like lesions, but no such correlation is suggested with EBV or CMV.

(ii) Fever of unknown origin. There were five patients who developed fevers of unknown origin. All five had serological evidence of CMV infection, including three who had primary infections. All antibody rises occurred around the time of the fever episode, or within 1 or 2 weeks after these episodes. Two also had serological rises against HSV, two had rises against EBV, one had a rise against measles, and one had rises against all three parainfluenza types. Four out of the five had >1% atypical lymphocytes in their blood smear. The one without atypical lymphocytes had a secondary bacterial infection. Four of these patients had signs of upper respiratory infection, two patients had

TABLE 3. *Frequency of serological responses to herpes-group viruses compared with responses to other viruses*

Virus	All patients			Initially seronegative patients			Initially seropositive patients		
	≥4× rise	<4× rise	Totals	Seroconversion	None	Totals	≥4× rise	<4× rise	Totals
Herpes group[a]	25[b] (38%)	41	66	3 (14%)	19	22	22 (50%)	22	44
Other[c]	12 (11%)	99	111	2 (20%)	8	10	10 (9.9%)	91	101
Totals	37	140	177	5	27	32	32	113	145
Significance	χ^2 (1) = 16.74, $P < 0.001$			Fisher's exact test, $P > 0.05$			χ^2 (1) = 26.37, $P < 0.001$		

[a] Includes CMV, HSV, and EBV.
[b] The numbers in this table represent events. See text.
[c] Includes rubella, measles, and parainfluenza types 1, 2, and 3.

TABLE 4. *Time of serological changes relative to time of transplantation*

Antigen	Changes[a]	Days after transplantation						
		1-30 fr[b] (%)	31-60 fr (%)	61-90 fr (%)	91-120 fr (%)	121-150 fr (%)	151-180 fr (%)	>180 fr (%)
CMV	Rises	0/8	1/17 (6)	4/17 (24)	2/10 (20)	0/10	3/9 (33)	0/8
	Falls	1/8 (13)	0/17	1/17 (6)	0/10	0/10	0/8	1/8 (13)
HSV	Rises	0/6	4/13 (31)	2/11 (18)	1/8 (13)	0/6	1/6 (17)	0/12
	Falls	0/6	0/13	1/11 (9)	0/8	0/6	0/6	0/12
EBV	Rises	1/8 (13)	3/15 (20)	1/15 (7)	0/8	0/11	0/8	1/10 (10)
	Falls	1/8 (13)	0/15	2/15 (13)	1/8 (13)	1/11 (9)	0/8	0/10
Other[c]	Rises	0/35	4/55 (7)	1/83 (1)	2/56 (4)	3/55 (5)	0/54	1/67 (1)
	Falls	0/35	1/55 (2)	1/83 (1)	0/56	3/55 (5)	5/54 (9)	3/67 (4)

[a] Rises and falls are changes in titer ≥fourfold occurring during period shown.
[b] fr, Fraction, number of ≥fourfold changes in titer in direction indicated/number of patients tested during indicated period against indicated antigen(s).
[c] Includes measles, rubella, and parainfluenzas 1, 2, and 3.

arthralgias, three had myalgia, and one had a cold sore.

(iii) Atypical lymphocytes. There were seven patients who showed atypical lymphocytes in their peripheral blood. Of these, six developed evidence of CMV serological change, including the three who were originally seronegative and hence presumably had primary infections. Only one patient had atypical lymphocytes without evidence of CMV infection, but this patient was only followed for 22 days postoperatively. Of the seven patients who developed atypical lymphocytes, three had serological rises against EBV and four did not.

(iv) Upper respiratory signs, liver function tests, and rejection. There were 11 patients with signs of upper respiratory infection, such as coryza and mild cough. Two of these had rises against parainfluenza viruses.

Five out of 15 patients had abnormal liver function tests, but only two of these had serological change against CMV.

There were 13 episodes of rejection, five of the very severe type. Only four of these had serological changes against CMV. We found no evidence that rejection was associated with CMV infection.

DISCUSSION

It is well known that CMV infections are frequent in renal transplant recipients. Infections with HSV (5) and varicella-zoster (7) have also been reported in such patients. However, it is not clear whether these changes represent: (i) enhanced susceptibility to exogenous and endogenous infections with these viruses; (ii) increases in antibody production during immunosuppression; (iii) expected variations in antibody titer in persons serially bled because of normal variation in titer and/or technical inaccuracies; or (iv) selected and specific reactivation of certain herpes-group viruses with subsequent antibody rise in the immunosuppressed transplant recipient. Our data favor the last interpretation. This effect is most clearly seen in Table 3, where only the initially seropositive patients are considered. Although the study group used was rather small, the use of 9 antigens and 4 to 10 serum samples per patient permitted an intensive study of the 23 patients

tested. The data are clear-cut with respect to CMV and HSV, in that the serological responses shown in Fig. 1 and 2 are outside the range of normal variation found when the single patient is followed over a long period of time and in that these responses seem to occur at a time related to renal transplantation. The rate of serological response to HSV in our patients is about twice that seen by Luby et al. (6) in transplant recipients but consistent with the experience of Fiala et al. (2), who observed reactivation of HSV in 35% of recipients.

There were 7 of 19 patients with a fourfold or greater rise in immunofluorescent antibody titer to Epstein-Barr viral capsid antigen. Although the reading of the test is subjective in nature, each was read independently by two persons, and each rise was confirmed in a repeat test. The results are also in accord with those of Spencer and Andersen (9), who reported EBV antibody rises in 3 of 30 renal transplant recipients in association with rises in CMV antibody in these same patients. Two of the three had fever. The results also in accord with those of Strauch et al. (10), who isolated EBV from the throats of 47% of 21 renal transplant recipients, 35% of 20 other patients receiving immunosuppressive drugs, and only 17% of healthy controls; however, the EBV antibody titer did not differ appreciably between excreters and non-excreters. Of five EBV antibody-positive patients who were non-excreters before transplantation, two excreted EBV afterwards. They believe the results were compatible with reactivation of latent EBV during immunosuppression. Recently, however, Fiala et al. (2) found increases in EBV antibody in only 1 of 61 renal transplant recipients in a study in which CMV infection occurred in 96%, herpes simplex in 35%, and varicella-zoster in 24%.

Five other virus antigens used (rubella, measles, and the parainfluenzas) can be considered together. Our data give no evidence that reactivation of these viruses as reflected by changes in antibody titer is a frequent occurrence in renal transplant recipients.

With respect to clinically recognizable disease, the evidence points to the importance of CMV infection, in particular to primary CMV infections. All three patients with primary CMV infections, two of whom were known to have received kidneys from seropositive donors (data not available on the third donor), developed symptomatic disease characterized by fever and atypical lymphocytosis; in some of them arthralgia, myalgia, and hepatosplenomegaly were manifested. The majority of reactivation infection with CMV appeared to be asymptomatic. The presence of cold sores corresponded only marginally with serological changes against HSV. Similarly, we could find no particular pattern of illness associated with serological rises to HSV, EBV, or other viral antigens.

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